Postharvest and Quality Management of Horticultural Products
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MOLECULAR PHYSIOLOGY STUDIES OF BANANA FRUIT RIPENING

Identification of candidate genes for improvement of fruit quality traits throughout breeding

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Abstract

Banana is a complex species and attempts to improve the fruit quality through breeding strategies are complex and difficult to set up. Identification of the main fruit quality traits and gaining further insight into the molecular mechanisms that govern their elaboration and their regulation are a key prerequisite for the development of strategies to improve banana quality traits like postharvest technology, breeding and/or biotechnology approaches. In this way, access to functional molecular markers derived from relevant genes associated with main fruit quality traits should therefore provide a valuable and helpful resource for the development of these strategies.

In the present study, we report the cloning and sequencing of genes that are differentially expressed during fruit ripening, as tools for functional genomic studies and putative molecular marker developments. Different molecular biology approaches have been used to isolate these ripening related-genes. They include cDNA amplification (RT- and RACE-PCR) and construction of complete and subtractive suppressive cDNA libraries (SSH). Sequencing and BLAST analysis of some of these isolated cDNA clones revealed that 205 of them presented a high homology with different genes in database. Many of the predicted proteins encoded by these genes are putatively involved in the regulation of gene expression, hormonal metabolism, hormonal-signal transduction, sugar metabolism and other ripening process. Among these 205 genes, 11 are still unclassified since presenting homology to unknown proteins of rice or *Arabidopsis*. Finally, thirteen additional clones were putatively novel, since they failed to match with database sequences.

Furthermore, expression of genes involved in some fruits quality traits was examined in order to identify those of which expression could be correlated with the related physiological processes and thus considered as candidates.

These genes will contribute to increase pools of public EST collections of banana, one of the weakest public EST collection among those of the most consumed fruits. Moreover our data led us to gain more insights into the banana ripening process and to suggest some candidates.

Keywords: Banana, Ethylene, Fruit, Musa, Ripening, finger drop, Gene cloning

MOLECULAR PHYSIOLOGY OF BANANA FRUIT RIPENING AND QUALITY IMPROVEMENT

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INTRODUCTION

- For fruit, quality traits including nutritional and organoleptic aspects can be considered as a source of biodiversity and increase the added value of the product and consequently the incomes of the producers

- Fruit Quality is a complex criteria (external and internal aspects as color, flavour, texture, aroma etc.) that is highly affected by both biotic and abiotic cues, some of them being antagonistic

INTRODUCTION

- Elaboration of fruit quality traits, mainly set up during ripening, involved number of complex physiological processes resulting from the coercitive action of different genes

- Banana fruit undergoes a ripening climacteric process characterized by a peak of respiration and a burst of an autocatalytic production of ethylene, concomitantly with molecular and biochemical changes leading to fruit ripening (peel degreening, aroma volatiles, sugar accumulation or fruit softening).

OBJECTIVES

- Evaluate the variability of quality traits within banana species
- Investigate the physiological mechanisms that govern the elaboration of these quality traits
- Generate molecular tools for improvement of banana fruit quality throughout conventional breeding

APPROACH

Characterization of banana fruit quality traits

Quality biodiversity

Identification of contrasted varieties

Development of molecular markers

Studies of Gene-Function-Phenotype relationship

Identification of candidate genes

Genetic studies

Breeding program

Biotechnology approach

Improvement of banana fruit quality traits

Understanding banana fruit ripening process

Getting genome resources from banana

Genes - antibodies

RESULTS

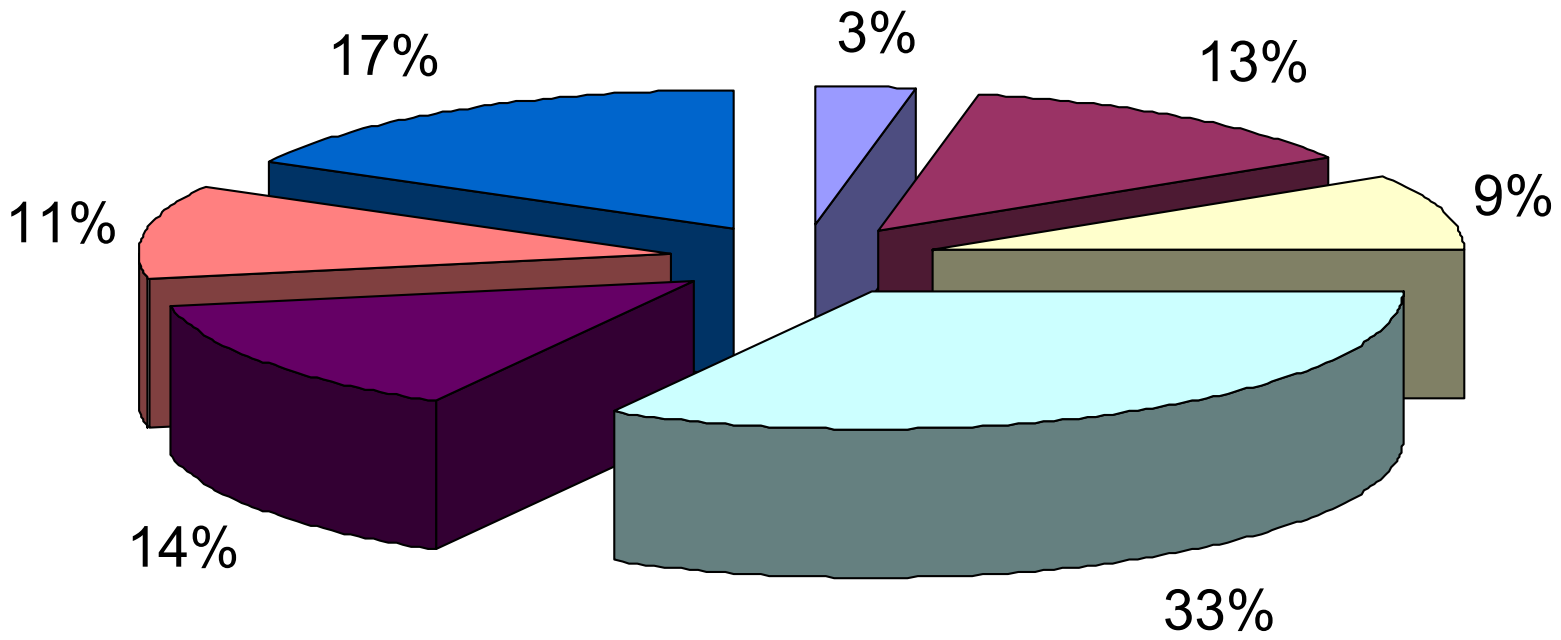
Characterization of banana quality traits (undergoing)

- A core collection of more than 30 different banana species was constituted and fruits are currently under characterization

- This collection includes cooking and dessert banana and, cultivated, wild types and hybrids cultivars

RESULTS

Genomic ressources



Distribution and frequency of putative functions of cDNA clones isolated from banana fruit

RESULTS

Genes-Functions Studies

- Three ripening processes involved in fruit quality traits elaboration are currently under investigation in order to identify the related candidate genes

  - Ethylene fruit responsiveness (ripening initiation process)
  - Finger drop phenomenon (commercial value of fruit)
  - Sucrose metabolism (sensory properties of fruit)

RESULTS

Ripening initiation

Name	Size (pb)	Accession n°	Blast best hits description
SSh2e08	224	DV270719	MADS-Box transcription factor
SSh2e08	497	DV270722	IAA-amino acid hydrolase
pCav44	1293	AF445195	Ethylene receptor (ERS-like) protein
pCav45	1676	AF445196	
AB266316	2210	AB266316	
MaEIL1	1817	DQ682615	EIN3-Like protein
MaEIL2/AB266318	1841	DQ682616	
MaEIL3/AB266319	1924	DQ682617	
MaEIL4/AB266320	2448	DQ682618	
AB266321	2313	AB266321	
pCav53	1925	Unregistered	CTR-Like protein

cDNA isolated from Cavendish fruit and putatively involved in ethylene fruit responsiveness and ripening initiation processes. AB266316 – 20 have been already published in Genbank database

RESULTS

ERS-like, EIN3 and EIN3-Like (EIL) protein

- ERS are transmembrane protein and EIN3/EIL protein are primary and nuclear transcription factors

- EIN3/EIL protein downstream of the ethylene receptor, redundantly as positive regulators of ethylene signaling

- EIN3/EIL and ERS proteins are encode by a small multigene family

- Regulation EIN3/EIL and ethylene receptor protein gene expression occurs at both mRNA and protein levels

RESULTS

Ripening initiation process

3 days after treatment

6 days after treatment

40 DAF

60 DAF

90 DAF

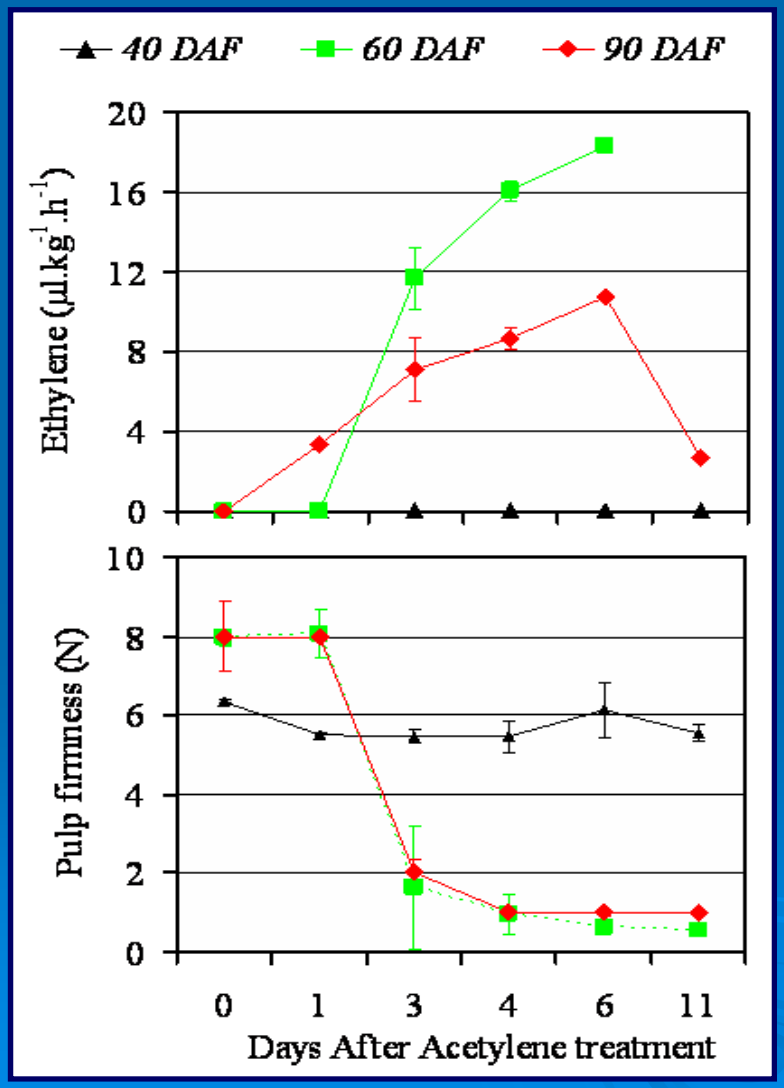
10^5 10^4 10^3 10^2 10^5 10^4 10^3 10^2

ppm of acetylene applied at 20°C

DAF: days after flowering

RESULTS

Ripening initiation process



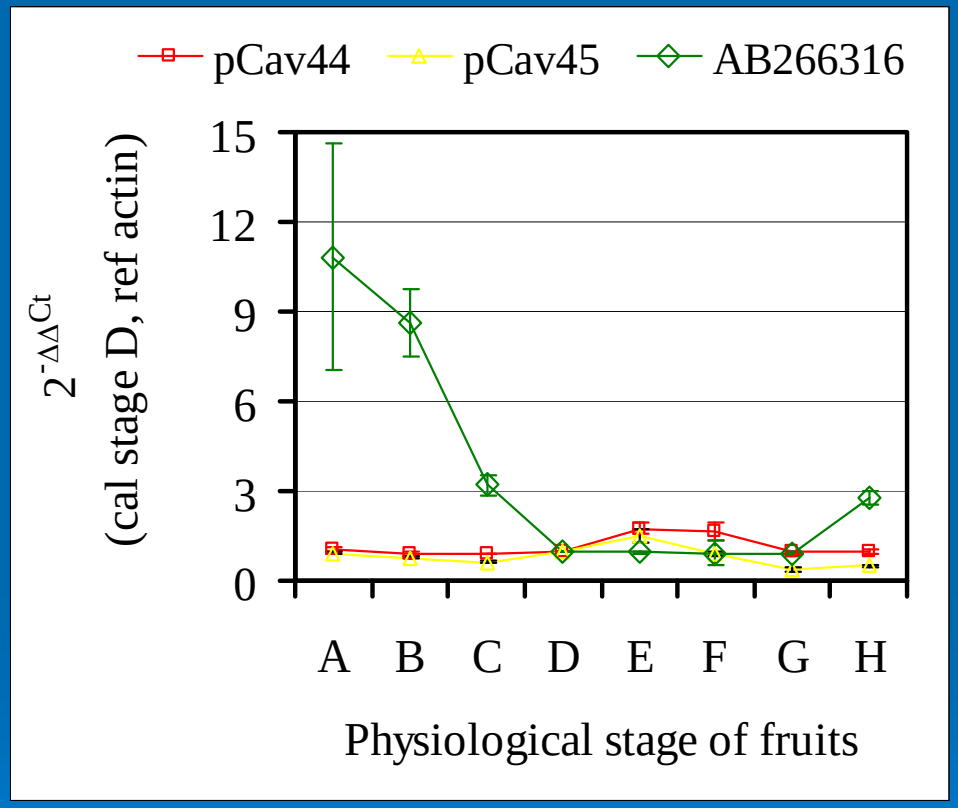
Ripening conditions: 20°C in air - ambiant humidity

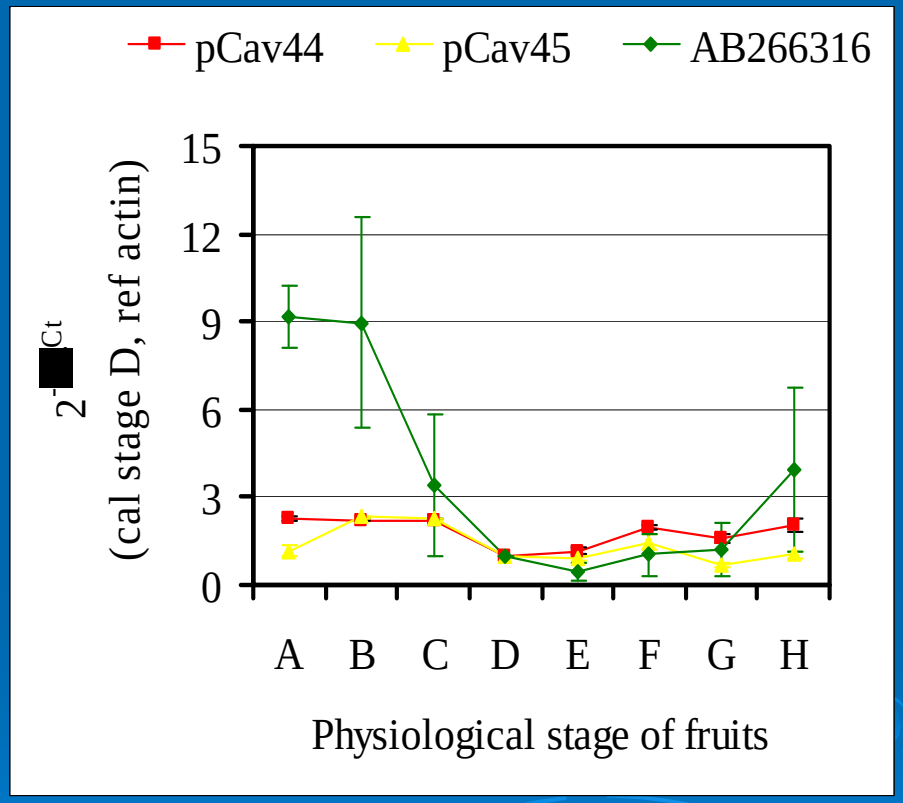
Ripening induction: 10^4 ppm of acetylene at 20°C

Banana fruit undergoes changes in ethylene responsiveness during green development stage and between 40, 60 and 90 days after flowering

RESULTS

ERS-Like gene expression



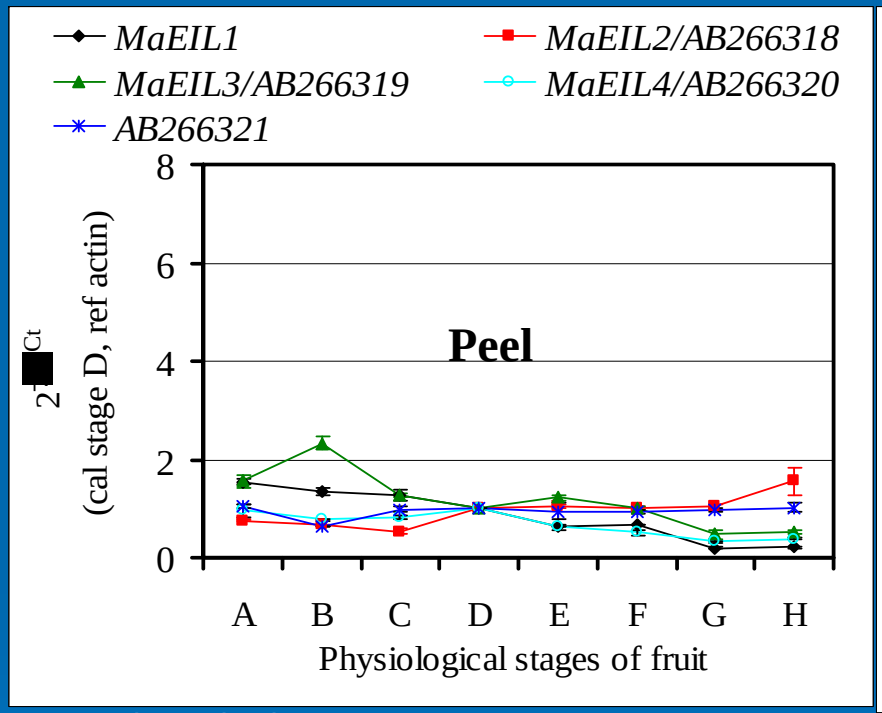


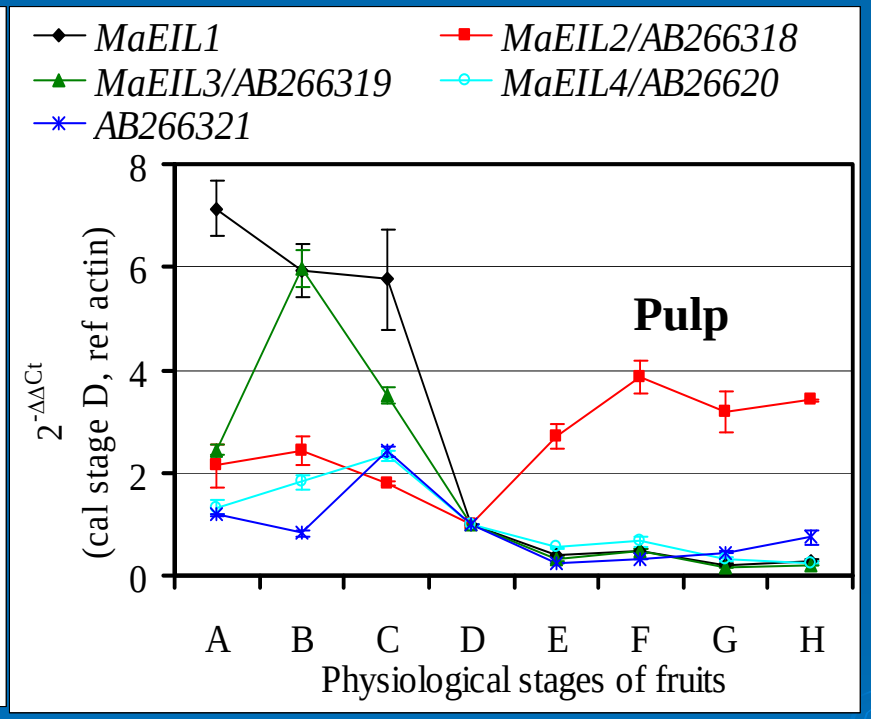
Expression of ethylene receptor genes during green development (A, B, C, D) and late ripening (E, F, G, H) stages of Cavendish banana indicated in the x-axis. ΔΔCt on the y-axis refers to the fold difference in gene expression to fruit taken at stage D (calibrator) and a housekeeping actin gene (reference). A = 30 DAF; B = 40 DAF; C = 60 DAF; D = 90 DAF; E, F, G and H = 90 DAF-fruits taken 1, 3, 6 and 11 days after initiation of ripening by exogenous acetylene treatment (10000ppm/20°C/24h), respectively

AB266316 considered as candidate for ripening initiation process

RESULTS

EIN3-Like gene expression





(Mbeguié-A-Mbéguié et al., Physiol Plant 133:435-448)

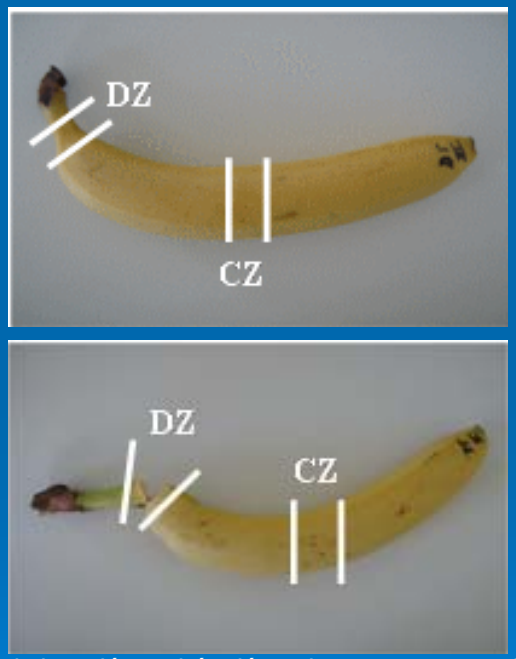
Expression of MaEIL genes during green development (A, B, C, D) and late ripening (E, F, G, H) stages of Cavendish banana indicated in the x-axis. ΔΔCt on the y-axis refers to the fold difference in gene expression to fruit taken at stage D (calibrator) and a housekeeping actin gene (reference). A = 30 DAF; B = 40 DAF; C = 60 DAF; D = 90 DAF; E, F, G and H = 90 DAF-fruits taken 1, 3, 6 and 11 days after initiation of ripening by exogenous acetylene treatment (10000ppm/20°C/24h), respectively.

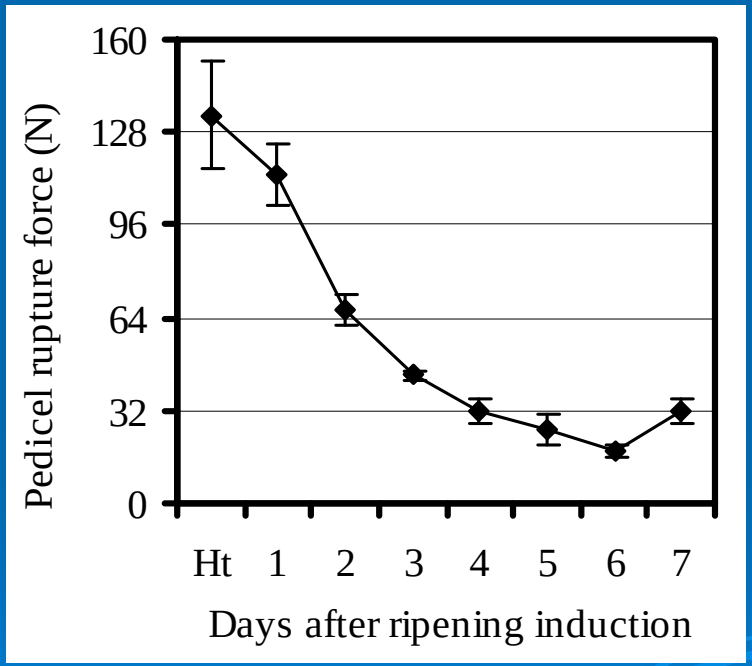
MaEIL1, MaEIL3/AB266319 and MaEIL2/AB266318 are putative candidates for ripening initiation process and control of late ripening process, respectively

RESULTS

Finger drop process – Physical measurement

CZ = Control Zone
DZ = Drop Zone





(Mbeguié-A-Mbéguié et al., J. Exp. Bot 60 (7):2021-2034)

Ripening conditions: 20°C in air - ambiant humidity

Ripening induction: 10^4 ppm of acetylene at 20°C

RESULTS

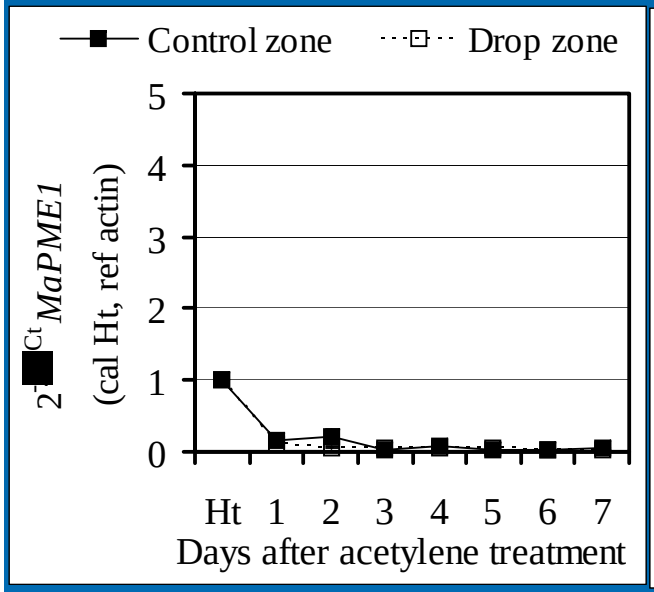
Finger drop process – Genomic resources

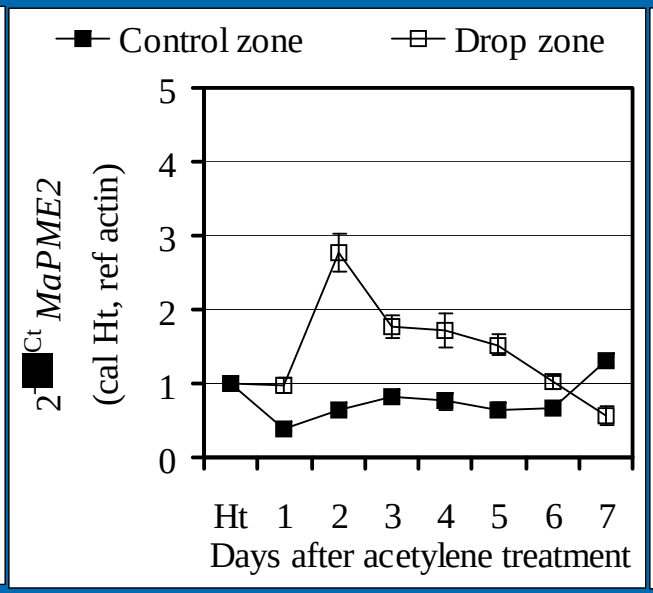
Genes	Number of genes isolated	Function
Pectin methylesterase (PME) [a]	3	Pectin degradation
Polygalacturonase (PG)[b]	4	
Pectate lyase (PEL)[c,d]	2	
Xyloglucan endotransglycosylase hydrolase (XTH)[a,e]	10	Xyloglucan metabolism
Expansin[f,g]	4	Cell wall loosening

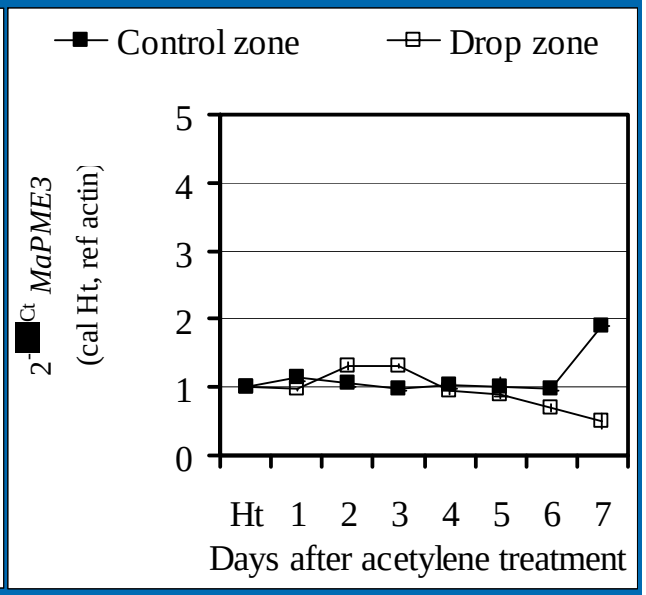
[a] *Mbeguié-A-Mbéguié et al., J. Exp. Bot 60 (7):2021-2034);* [b] *Asif and Nath. Plant Physiol. Bioch. 43:177–184.*
[c] *Dominguez-Puigjaner et al., Plant Physiol. 114:1071–1076;* [d] *Pua et al., Physiol. Plant. 113: 92–99.*
[e] *Lu et al., Acta Botanica Sinica 46, 355–362;* [f] *Trivedi and Nath. Plant Science 167, 1351–1358.*
[g] *Sane et al., Post-harvest Biol. Technol. 45, 184–192.*

RESULTS

Finger drop process – MaPME Genes expression







MaPME2 can be considered as candidate

Other candidates :

MaPG4,

MaEXP1, 4 and 5

MaXTH1, 2, 6, 8 and 9

MaPEL1 and 2

CONCLUSIONS

- Elaboration of quality traits of banana fruit and as probably other tropical fruits implies a complex physiological processes and different genes.

- Therefore improvement of genomique resources quality as well as getting more information on their function on fruit quality traits elaboration remain a challenge.

PROSPETS

Investigations are currently undergoing at different levels on the candidate genes identified in this study:

- Getting more information on MaEIL1, 2 and 3 protein.

- Getting more information on the relationship between environmental cues (cultural practices, postharvest technologies on candidate gene expression

- Exploiting of banana collection available at CIRAD (Guadeloupe, FWI) to examine the relationship between the structural genomique sequences and expression.

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